510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k033706

B. Analyte:
Homocysteine

C. Type of Test:
Quantitative enzymatic colorimetric assay

D. Applicant:
Polymedco, Inc.

E. Proprietary and Established Names:
Polymedco Homocysteine Test for the Poly-Chem and Hitachi Chemistry Systems;
Polymedco Assayed Homocysteine Level 1 and Level 2 Controls;
Polymedco Homocysteine Level 1 and Level 2 Calibrators

F. Regulatory Information:

1. Regulation section:
   21 CFR 862.1377; 21 CFR 862.1660; 21 CFR 862.1150
2. Classification:
   Class II; Class I; Class II
3. Product Code:
   LPS; JJX; JIT
4. Panel:
   75 Clinical Chemistry

G. Intended Use:

1. Intended Use(s)
   The Polymedco Homocysteine Assay is intended for the quantitative *in vitro* determination of total homocysteine in serum and plasma on automated clinical chemistry analyzers.

   The Homocysteine Calibrators are a device intended for medical purposes for use with the Polymedco Homocysteine assay to establish points of reference that are used in determination of values in the measurement of homocysteine in human serum or plasma.

   The Polymedco Assayed Homocysteine Level 1 and Level 2 Controls are intended for use as an assayed quality control material to monitor the precision and accuracy of the test for homocysteine.

2. Indication(s) for use:

   The Polymedco Homocysteine Test is intended for the quantitative in vitro determination of total homocysteine in serum and plasma on automated clinical chemistry analyzers. This device can assist in the diagnosis and

treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

3. Special condition for use statement(s):
   Prescription Use Only
4. Special instrument Requirements:
   This assay has been validated on the Roche Hitachi and Polymedco analyzers.

H. Device Description:

The assay consists of **R1A reagent** (dithioreitol, (10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)phenthiazine), and homocysteine methyltransferase; **R1B reagent** ((dithioreitol and (10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)phenthiazine); **R2 reagent** (D-amino acid oxidase, peroxidase); **R1 diluent** (D-methionine methylsulfonium); and **R2 diluent** (N-erylymaleimide).

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Abbott IMx Homocysteine
2. Predicate K number(s):
   K992858
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Quantitative Measurement of Homocysteine	Same
Indications for Use	Diagnosis and Treatment of Hyperhomocysteinemia and Homocystinuria.	Same
Matrices	Serum and Plasma	Same
Assay Reactions	Enzymatic Redox Reaction	Same
Differences		
Item	**Device**	**Predicate**
Endpoint	Colored End Product	Intensity of Polarized Fluorescent Light
Calibrators	D-methionine , Two levels	S-adenosyl-L-homocysteine, Five levels
Controls	L-homocysteine, Two levels	L-homocysteine, Three levels
Reportable Range	Polychem: 2.01-126 µmol/L Hitachi: 1.35 – 118 µmol/L	0.8 – 500 µmol/L (with auto-dilution)

J. Standard/Guidance Document Referenced (if applicable):

None referenced by sponsor

K. Test Principle:

The sample is reduced by dithiothreitol (DTT) to generate free Hcy and simultaneously homocysteine methyltransferase (HCMT) transfers the methyl group of D-methionine methylsulfonium (DMMS) to Hcy, resulting in the generation of L-Methionine and D-Methionine (D-Met). In the second step, the generated D-Met is oxidized by D-amino acid oxidase (DAO) along with the production of hydrogen peroxide, followed by the oxidation of DA-67 (10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)phenthiazine) to yield methylene blue. This assay system contains N-erylymaleimide (NEM) to capture the thiol group of the remaining DTT, which inhibits the oxidation of DA-67. Some D-amino acids are also measured in addition to Hcy. To avoid interference, the background level of D-amino acids in each sample is also determined and subtracted.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra assay precision was evaluated separately on the Hitachi 717 and Polychem systems by assaying 20 replicates of three different concentrations in a single analytical run. Results were as follows:

Poly-Chem Analyzer	Level 1 (µmol/L)	Level 2 (µmol/L)	Level 3 (µmol/L)
N	20	20	20
Mean	10.2	17.1	34.6
SD	0.31	0.45	1.02
CV% (acceptance criteria <5%)	3.07	2.61	2.96
Hitachi Analyzer 717	Level 1 (µmol/L)	Level 2 (µmol/L)	Level 3 (µmol/L)
N	20	20	20
Mean	10.7	31.7	76.6
SD	0.36	0.4	0.5
CV% (acceptance criteria <5%)	3.34	1.23	0.67

Inter assay precision was evaluated separately on the Hitachi 717 and Polychem systems by assaying duplicates of three different concentrations in each of ten different runs over ten days. Results were as follows:

Poly-Chem Analyzer	Level 1 (µmol/L)	Level 2 (µmol/L)	Level 3 (µmol/L)
Days	10	10	10
N	20	20	20
Mean	10.7	30.9	76.2
SD	0.36	0.66	1.08
CV% (acceptance criteria <10%)	3.36	2.12	1.42
Hitachi Analyzer 717	Level 1 (µmol/L)	Level 2 (µmol/L)	Level 3 (µmol/L)
Days	10	10	10
N	20	20	20
Mean	10.3	30.4	75.9
SD	0.4	0.5	1.1
CV% (acceptance criteria <5%)	4.28	1.79	1.45

b. *Linearity/assay reportable range:*
Linearity/calibration curve fit data was generated for a pool that spans the linear range of the test. Serial dilution sets were prepared using 0.9% saline solution and made up fresh and assayed with each of two calibrated runs. The results at each level of analyte were averaged and the linear fit assessed for levels 1-3 and then for levels 2-4. The linearity claim is based on a percent deviation of < 5% at the two highest analyte concentrations. The linearity evaluation was performed on Polymedco Poly-Chem and Hitachi 717 Chemistry Analyzers with the following results:

Poly-Chem Linear fit parameters for levels 1-3:
y = 0.98x + 3
r = 0.973
Poly-Chem Linear fit parameters for levels 2-4:
y = 1.14x - 3
r = 0.989

Hitachi 717 Linear fit parameters for levels 1-3:
y = 1.01x -1
r = 1.000
Hitachi 717 Linear fit parameters for levels 2-4:
y = 0.98x –0.4
r = 0.999

c. *Traceability (controls, calibrators, or method):*
The sponsor states that the value assignment of the calibrators and controls includes testing by an HPLC method for homocysteine.

d. *Detection limit:*
The criteria for determining functional sensitivity was an inter-assay CV of 20% (n=10) with the mean of the replicates within 10% of the target value. Using these criteria, the functional sensitivity on the Poly-Chem analyzer was determined to be 2.0 µmol/L and on the Hitachi 717 was determined to be 1.35 µmol/L.

e. *Analytical specificity:*
Three sample pools were tested at Homocysteine concentrations of approximately 5, 11, and 21 umol/L for interference by the substances listed below. Less than 10% interference was observed for the listed concentrations.

POTENTIAL INTERFERENT	CONCENTRATION
S-(5'Adenosyl)-L-Methionine	0.05 mmol/L
L-Cysteine	100 mmol/L
L-Cystathionine	0.50 mmol/L
Adenosine	5.0 mmol/L

Glutathione	100 mmol/L
Total Protein	4.0 g/dL
Red Blood Cells	5%

In addition, Hemoglobin was evaluated in three samples that contained 9.6, 16.5 and 27.0 µmol/L of Homocysteine and it was found that a concentration of 250 mg/dl did not interfere with the measurement of homocysteine.

Triglyceride was evaluated in three samples that contained 6.4, 14.5, and 27.1 µmol/L Homocysteine and it was found that a concentration of 311 mg/dl did not interfere with the measurement of homocysteine.

f. Assay cut-off:
N/A

2. Comparison studies:

a. Method comparison with predicate device:
A method comparison study was performed comparing the PolyMedco assay on the Poly-Chem analyzer vs. the predicate device. Eighty-seven serum samples ranging in concentration from 6.9 to 51.6 µmol/L were compared, producing the following results:

PolyChem = 0.90 Predicate + 0.14; r = 0.976

The Poly-Chem analyzer was also compared to an HPLC method. Forty serum samples ranging in concentration from 6.1 to 79.8 µmol/L were compared, producing the following results:

PolyChem = 0.89 HPLC + 2.02; r = 0.999

A method comparison study was also performed comparing the PolyMedco assay on the Hitachi 717 analyzer vs. the HPLC method. Forty serum samples ranging in concentration from 5.3 to 83.7 µmol/L were compared, producing the following results:

Hitachi 717 = 1.07 HPLC – 1.80; r = 0.999

b. Matrix comparison:
Matrix comparison studies were done comparing serum with EDTA and heparin plasma. In both studies 40 paired samples ranging from approximately 5 to 90 µmol/L were analyzed, with the following results:
EDTA plasma = 1.03 serum + 0.3; r = 0.999
Heparin plasma = 1.00 serum – 0.2; r = 0.999

3. Clinical studies:
    a. *Clinical sensitivity:*
    N/A
    b. *Clinical specificity:*
    N/A
    c. *Other clinical supportive data (when a and b are not applicable):*

4. Clinical cut-off:
    N/A
5. Expected values/Reference range:
    The sponsor has used reference ranges cited from the medical literature:

    "A cut off of 15.0 µmol/L is generally associated for risk of atherosclerotic disease. Cutoffs associated with nutritional status are listed in the table below."

	12-19 Years of Age	≥ 60 Years of Age	Cut-off for High Levels
Male	4.3-9.9 µmol/L	5.9-15.3 µmol/L	≥11.4 µmol/L
Female	3.3-7.2 µmol/L	4.9-11.6 µmol/L	≥10.4 µmol/L

    The sponsor recommends that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population it serves.

M. Conclusion:

Based upon the information provided for the file, I recommend that the Polymedco Homocysteine Test be found substantially equivalent to the predicate device.